

PATENT APPLICATION

of

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for

SYNERGISTIC MICROBICIDAL COMBINATIONS

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SYNERGISTIC MICROBICIDAL COMBINATIONS

BACKGROUND

This invention relates to synergistic microbicidal combinations and, in particular, to synergistic microbicidal combinations of 2-methyl-3-isothiazolone with one or more selected commercial microbicides, where the resulting composition is substantially free of halogenated 3-isothiazolone.

Kathon™ CG biocide (3/1 ratio of 5-chloro-2-methyl-3-isothiazolone to 2-methyl-3-isothiazolone) and Neolone™ 950 bactericide (2-methyl-3-isothiazolone) are highly effective preservatives and may be used in combination with a variety of commercially available preservatives to prevent microbial contamination in personal care applications, such as cosmetics and toiletries (see "Kathon™ CG Cosmetic and Toiletry Preservative" Bulletin CS-663, September 1997, and "Neolone™ 950 Preservative for Personal Care" Bulletin CS-707, May 2001, Rohm and Haas Company, Philadelphia, PA). U.S. Patent No. 5,591,759 discloses the use of 1,2-dibromo-2,4-dicyanobutane to stabilize isothiazolone mixtures of 5-chloro-2-methyl-3-isothiazolone/2-methyl-3-isothiazolone (9/1 ratio). Patent application WO 01/50855 discloses preservative formulations for extending the lifetime of cut flowers based on preservative formulations containing acrylic copolymer additives, saccharide derivatives and antimicrobial mixtures, including those based on 2-methyl-3-isothiazolone in the presence of citric acid/citrates.

Many other microbicidal agents are known. These are commercially available for the control of microorganisms in a variety of loci. Sometimes, many microbicides cannot provide effective antimicrobial control even at high use concentrations due to weak activity against certain microorganisms. Without effective microorganism control, loss of product, inferior product, production time loss, health hazard and other problems may occur in the locus treated. There is a need for a method to control various microorganisms that does not rely on high use levels of combinations of different microbicidal agents, but still provides effective overall control of the microorganisms that is both quick and long lasting.

The problem addressed by the present invention is to overcome deficiencies of previous microbicide combinations by providing a combination of microbicidal agents that is more effective than the individual microbicides used alone and that can be used at lower overall levels while providing efficacy
5 similar to the original individual microbicide levels.

STATEMENT OF INVENTION

The present invention provides a microbicidal composition comprising a synergistic mixture, the first component of which is 2-methyl-3-isothiazolone, and the second component of which is one or more commercial microbicides
10 selected from the group consisting of benzoic acid, sorbic acid, 1,2-dibromo-2,4-dicyanobutane, 1,3-dimethylol-5,5-dimethylhydantoin, phenoxyethanol, zinc pyrithione and climbazole; wherein the ratio of the first component to the second component is from 1/0.001 to 1/1000; and wherein the composition is substantially free of halogenated 3-isothiazolone.

15 In another embodiment the present invention provides a microbicidal composition comprising a synergistic mixture, the first component of which is 2-methyl-3-isothiazolone, and the second component of which is one or more commercial microbicides selected from the group consisting of citric acid and benzyl alcohol; wherein the ratio of the first component to the second component
20 is from 1/8 to 1/24 when the second component is citric acid; wherein the ratio of the first component to the second component is from 1/0.13 to 1/32 or from 1/80 to 1/1600 when the second component is benzyl alcohol; and wherein the composition is substantially free of halogenated 3-isothiazolone.

The present invention further provides a method of inhibiting the growth
25 of microorganisms in a locus comprising introducing to, at or on, the locus a microorganism inhibiting amount of the aforementioned synergistic mixture; and wherein the amount of synergistic mixture is from 0.1 to 10,000 parts per million active ingredient.

In a preferred embodiment the present invention provides a method of
30 inhibiting the growth of microorganisms as described above wherein the microorganisms are selected from one or more of bacterial and fungal microorganisms.

DETAILED DESCRIPTION

We have discovered that 2-methyl-3-isothiazolone (**MI**) may be combined with selected commercial microbicides to provide enhanced microbicidal efficacy at a combined active ingredient level lower than that of the combined individual 3-isothiazolone or commercial microbicide effective use levels, where the resulting composition is substantially free of halogenated 3-isothiazolone. Preferably the microbicidal compositions are also substantially free of metal salt stabilizers, such as nitrate or magnesium salts; these "salt-free" microbicidal compositions are especially useful to protect personal care compositions against microbial contamination.

It has been discovered that mixtures of **MI** with one or more of the commercial microbicides of the present invention in a ratio of 1/0.001 to 1/1000 result in synergistic microbicidal activities against a wide range of microorganisms. Synergy occurs when the disruptive interaction on the organisms treated by the two compounds together is greater than the sum of such interactions of both compounds when used alone. Such synergy does not arise from the expected activity of the components when added together.

As used herein, the following terms have the designated definitions, unless the context clearly indicates otherwise. The term "microbicide" refers to a compound capable of inhibiting the growth of or controlling the growth of microorganisms at a locus; microbicides include bactericides, fungicides and algacides. The term "microorganism" includes, for example, fungi (such as yeast and mold), bacteria and algae. The term "locus" refers to an industrial system or product subject to contamination by microorganisms. The following abbreviations are used throughout the specification: ppm = parts per million by weight (weight/weight), mL = milliliter, ATCC = American Type Culture Collection, and MIC = minimum inhibitory concentration. Unless otherwise specified, ranges listed are to be read as inclusive and combinable, temperatures are in degrees centigrade (°C), and references to percentages (%) are by weight. "Salt-free" means that the composition contains zero or up to 0.5%, preferably zero or up to 0.1%, and more preferably zero or up to 0.01%, of metal salt, based on weight of the composition.

The microbicidal compositions of the present invention are substantially free of halogenated 3-isothiazolone; that is, zero or up to 3%, preferably zero or up to 1% and more preferably zero or up to 0.5%, of halogenated 3-isothiazolone may be present, based on combined weight of halogenated 3-isothiazolone and 2-methyl-3-isothiazolone. Microbicidal compositions dependent on the presence of halogenated 3-isothiazolone are susceptible to chemical degradation and may require additional stabilizer components, such as the aforementioned metal salt stabilizers; salt stabilizers sometimes create unacceptable properties in finished formulations. For this reason it is desirable to provide microbicide formulations substantially free of halogenated 3-isothiazolone, but that still provide the degree of antimicrobial protection provided by the halogenated 3-isothiazolones; such are the microbicidal compositions of the present invention that are based on 2-methyl-3-isothiazolone, which do not require metal stabilizers.

MI may be used in the synergistic mixtures of the present invention "as is" or may first be formulated with a solvent or a solid carrier. Suitable solvents include, for example, water; glycols, such as ethylene glycol, propylene glycol, diethylene glycol, dipropylene glycol, polyethylene glycol, and polypropylene glycol; glycol ethers; alcohols, such as methanol, ethanol, propanol, phenethyl alcohol and phenoxypropanol; ketones, such as acetone and methyl ethyl ketone; esters, such as ethyl acetate, butyl acetate, triacetyl citrate, and glycerol triacetate; carbonates, such as propylene carbonate and dimethyl carbonate; and mixtures thereof. It is preferred that the solvent is selected from water, glycols, glycol ethers, esters and mixtures thereof. Suitable solid carriers include, for example, cyclodextrin, silicas, diatomaceous earth, waxes, cellulosic materials and charcoal. It is preferred that **MI** is formulated in water.

The second microbicide components of the combinations of the present invention are well-known and generally commercially available microbicides, and include benzoic acid, citric acid, sorbic acid, 1,2-dibromo-2,4-dicyanobutane, 1,3-dimethylol-5,5-dimethylhydantoin, phenoxyethanol, benzyl alcohol, zinc pyrithione and climbazole. The ratio of **MI** to the second microbicide component is typically from 1/0.001 to 1/1000, and preferably from 1/0.05 to 1/100. These microbicides may be used in the synergistic mixtures of the present invention "as

is" or may first be formulated together with a suitable solvent, a solid carrier or as a dispersion. Suitable solvents and solid carriers are those described above for **MI**.

When a water-insoluble second microbicide component is formulated in a solvent, the formulation may optionally contain surfactants. When such formulations contain surfactants, they are generally in the form of emulsive concentrates, emulsions, microemulsive concentrates, or microemulsions. Emulsive concentrates form emulsions upon the addition of a sufficient amount of water. Microemulsive concentrates form microemulsions upon the addition of a sufficient amount of water. Such emulsive and microemulsive concentrates are generally well known in the art; it is preferred that such formulations are free of surfactants. U.S. Patent No. 5,444,078 may be consulted for further general and specific details on the preparation of various microemulsions and microemulsive concentrates.

Any formulation of **MI** may be used with any formulation of the second microbicide component in the synergistic mixtures of the present invention. When both the **MI** and the second microbicide component are each first formulated with a solvent, the solvent used for **MI** may be the same as or different from the solvent used to formulate the other commercial microbicide. It is preferred that the two solvents are miscible. In the alternative, the **MI** and the other microbicide may be combined directly and then a solvent added to the mixture.

Those skilled in the art will recognize that the **MI** and the second microbicide component of the present invention may be added to a locus sequentially, simultaneously, or may be combined before being added to the locus. It is preferred that the **MI** and the second microbicide component be added to a locus simultaneously or combined prior to being added to the locus. When the microbicides are combined prior to being added to a locus, such combination may optionally contain adjuvants, such as, for example, solvent, thickeners, anti-freeze agents, colorants, sequestrants (such as ethylenediamine-tetraacetic acid, ethylenediaminedisuccinic acid, iminodisuccinic acid and salts

thereof), dispersants, surfactants, stabilizers, scale inhibitors and anti-corrosion additives.

The microbicidal compositions of the present invention can be used to inhibit the growth of microorganisms by introducing a microbicidally effective
5 amount of the compositions onto, into, or at a locus subject to microbial attack. Suitable loci include, for example: cooling towers; air washers; boilers; mineral slurries; wastewater treatment; ornamental fountains; reverse osmosis filtration; ultrafiltration; ballast water; evaporative condensers; heat exchangers; pulp and paper processing fluids; plastics; emulsions; dispersions; paints; latexes;
10 coatings, such as varnishes; construction products, such as mastics, caulks, and sealants; construction adhesives, such as ceramic adhesives, carpet backing adhesives, and laminating adhesives; industrial or consumer adhesives; photographic chemicals; printing fluids; household products, such as bathroom and kitchen cleaners; cosmetics; toiletries; shampoos; soaps; detergents;
15 industrial cleaners; floor polishes; laundry rinse water; metalworking fluids; conveyor lubricants; hydraulic fluids; leather and leather products; textiles; textile products; wood and wood products, such as plywood, chipboard, flakeboard, laminated beams, oriented strandboard, hardboard, and particleboard; petroleum processing fluids; fuel; oilfield fluids, such as injection
20 water, fracture fluids, and drilling muds; agriculture adjuvant preservation; surfactant preservation; medical devices; diagnostic reagent preservation; food preservation, such as plastic or paper food wrap; pools; and spas.

Preferably, the microbicidal compositions of the present invention are used to inhibit the growth of microorganisms at a locus selected from one or more
25 of emulsions, dispersions, paints, latexes, household products, cosmetics, toiletries, shampoos, soaps, detergents and industrial cleaners. In particular, the microbicidal compositions are useful in personal care applications, such as hair care (for example, shampoo and dyes) and skin care (for example, sunscreens, cosmetics, soaps, lotions and creams) formulations.

30 When the synergistic compositions of the present invention are used in personal care compositions, the formulated compositions may also comprise one or more ingredients selected from UV radiation-absorbing agents, surfactants,

rheology modifiers or thickeners, fragrances, moisturizers, humectants, emollients, conditioning agents, emulsifiers, antistatic aids, pigments, dyes, tints, colorants, antioxidants, reducing agents and oxidizing agents.

Suitable UV radiation-absorbing agents (including chemical absorbers and
 5 physical blockers) include, for example, oxybenzone, dioxybenzone, sulisobenzene, menthyl anthranilate, para-aminobenzoic acid, amyl para-dimethylaminobenzoic acid, octyl para-dimethylaminobenzoate, ethyl 4-bis(hydroxypropyl)aminobenzoate, polyethylene glycol (PEG-25) para-aminobenzoate, diethanolamine para-methoxycinnamate, 2-ethoxyethyl
 10 para-methoxycinnamate, ethylhexyl para-methoxycinnamate, octyl para-methoxycinnamate, isoamyl para-methoxycinnamate, 2-ethylhexyl-2-cyano-3,3-diphenylacrylate, 2-ethylhexyl salicylate, homomenthyl salicylate, glyceryl aminobenzoate, triethanolamine salicylate, digalloyl trioleate, lawsone with dihydroxyacetone, 2-phenylbenzimidazole-5-sulfonic acid, 4-methylbenzylidene
 15 camphor, avobenzene, titanium dioxide and zinc oxide. Alternatively, UV radiation-absorbing agents such as triazines, benzotriazoles, vinyl group-containing amides, cinnamic acid amides and sulfonated benzimidazoles may also be used.

Suitable surfactants include, for example, nonionic, anionic, cationic and
 20 amphoteric surfactants and mixtures thereof; such as PPG 28 Buteth 35, PEG 75 lanolin, perfluoropolymethyl isopropyl ether, octoxynol-9, PEG-25 hydrogenated castor oil, polyethylene terephthalate, polyethylene glycol 25 glyceryl trioleate, oleth-3 phosphate, PPG-5-ceteth-10 phosphate, PEG-20 methyl glucose ether, glycereth-7-triacetate and N-alkyl substituted lactam (such as N-octyl
 25 pyrrolidone).

Suitable thickeners or rheology modifiers include, for example, hydrophobically modified nonionic ethoxylated urethanes, polycarboxylic acid thickeners such as acrylates/steareth-20 methacrylate copolymer, carbomers, acrylates copolymer and acrylates C₁₀₋₃₀ alkyl acrylate crosspolymer.

30 The personal care compositions improved by the method of this invention include, for example:

(a) hair care formulations, including shampoos, hair dyes, hair conditioners, gels, mousses and hair sprays; and

(b) skin care and nail care formulations, including nail coatings, cosmetics, astringents, depilatories, facial make-up formulations, sunscreens and
5 sunblocks, premoistened wipes, hand creams, hand and body soaps, and hand and body lotions.

The cosmetic formulations typically contain water, film forming materials, emulsifiers, softeners, emollients, oils, stabilizers, thickeners, neutralizers, perfume, colorants, pigments and combinations thereof. The sunscreen
10 formulations typically contain UV radiation-absorbing agents, water, film forming materials, emulsifiers, emollients, waterproofing agents, oils, stabilizers, thickeners, preservatives, perfume, colorants, insecticides, humectants and combinations thereof.

Optionally, other additives, such as additional film forming agents,
15 plasticizers, antifoaming agents, leveling aids, excipients, vitamins, natural extracts, proteins, sequestrants, dispersants, antioxidants, suspending agents and solvents may be added to the personal care formulations described above. Suitable solvents include, for example, C₁-C₁₂ straight or branched chain alcohols such as ethanol, isopropanol, or propanol; alkyl esters such as ethyl
20 acetate; ketones; and combinations thereof.

The specific amount of the synergistic combinations necessary to inhibit or control the growth of microorganisms in a locus depends upon the particular compounds in the combination and particular locus to be protected. Typically, the amount of the synergistic combinations of the present invention to control
25 the growth of microorganisms in a locus is sufficient if it provides from 0.1 to 10,000 ppm active ingredient of the synergistic mixture in the locus. It is preferred that the synergistic mixture be present in an amount of 0.5 to 5000 ppm, and more preferably from 1 to 3000 ppm, in the locus.

Microorganisms evaluated for effectiveness of control by the compositions
30 of the present invention include bacterial (*P. aeruginosa* and *S. aureus*) and

fungal (*A. niger* and *C. albicans*) microorganisms, in particular Gram (–) bacteria and Gram (+) bacteria, as well as yeast and mold.

Combinations of **MI** with sorbic acid, 1,2-dibromo-2,4-dicyanobutane, 1,3-dimethylol-5,5-dimethylhydantoin or phenoxyethanol were particularly effective against both Gram (–) and Gram (+) bacteria. Combinations of **MI** with benzoic acid, zinc pyrithione or benzyl alcohol were particularly effective against Gram (–) bacteria. Combinations of **MI** with citric acid or climbazole were particularly effective against Gram (+) bacteria. Combinations of **MI** with phenoxyethanol, zinc pyrithione or benzyl alcohol were particularly effective against both yeast and mold. Combinations of **MI** with benzoic acid, 1,2-dibromo-2,4-dicyanobutane or climbazole were particularly effective against yeast. Combinations of **MI** with sorbic acid were particularly effective against mold. Combinations of **MI** with benzoic acid, sorbic acid, 1,2-dibromo-2,4-dicyanobutane, phenoxyethanol, zinc pyrithione, climbazole or benzyl alcohol were effective against both bacterial and fungal microorganisms.

When benzoic acid is used in the synergistic mixtures of the present invention, it is preferred that the ratio of **MI** to benzoic acid is from 1/0.1 to 1/100 and more preferably from 1/0.13 to 1/67. When citric acid is used in the synergistic mixtures of the present invention, it is preferred that the ratio of **MI** to citric acid is from 1/8 to 1/40 and more preferably from 1/8 to 1/24. When sorbic acid is used in the synergistic mixtures of the present invention, it is preferred that the ratio of **MI** to sorbic acid is from 1/2 to 1/150, more preferably from 1/4 to 1/133 and most preferably from 1/4 to 1/67. When 1,2-dibromo-2,4-dicyanobutane is used in the synergistic mixtures of the present invention, it is preferred that the ratio of **MI** to 1,2-dibromo-2,4-dicyanobutane is from 1/0.4 to 1/100. When 1,3-dimethylol-5,5-dimethylhydantoin is used in the synergistic mixtures of the present invention, it is preferred that the ratio of **MI** to 1,3-dimethylol-5,5-dimethylhydantoin is from 1/0.05 to 1/100 and more preferably from 1/0.06 to 1/80. When phenoxyethanol is used in the synergistic mixtures of the present invention, it is preferred that the ratio of **MI** to phenoxyethanol is from 1/1 to 1/1000 and more preferably from 1/2 to 1/800.

When zinc pyrithione is used in the synergistic mixtures of the present invention, it is preferred that the ratio of **MI** to zinc pyrithione is from 1/0.001 to 1/20 and more preferably from 1/0.0013 to 1/13. When climbazole is used in the synergistic mixtures of the present invention, it is preferred that the ratio of **MI** to climbazole is from 1/0.03 to 1/30 and more preferably from 1/0.05 to 1/24. When benzyl alcohol is used in the synergistic mixtures of the present invention, it is preferred that the ratio of **MI** benzyl alcohol is from 1/0.13 to 1/32 or from 1/80 to 1/1600 and more preferably from 1/80 to 1/400.

Some embodiments of the invention are described in detail in the following Examples. All ratios, parts and percentages are expressed by weight unless otherwise specified, and all reagents used are of good commercial quality unless otherwise specified. Abbreviations used in the Examples and Tables are listed below with the corresponding descriptions:

15	MI = 2-methyl-3-isothiazolone SI = synergy index MIC = minimum inhibitory concentration
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EXAMPLES

The synergism of the combinations of the present invention was demonstrated by testing a wide range of concentrations and ratios of the compounds.

Synergism was determined by an industrially accepted method described by Kull, F.C.; Eisman, P.C.; Sylwestrowicz, H.D. and Mayer, R.L., in Applied Microbiology 9:538-541 (1961), using the ratio determined by the formula:

25

$$Q_a/Q_A + Q_b/Q_B = \text{Synergy Index ("SI")}$$

wherein:

30	Q_A = concentration of compound A (first component) in ppm, acting alone, which produced an end point (MIC of Compound A). Q_a = concentration of compound A in ppm, in the mixture, which produced an end point. Q_B = concentration of compound B (second component) in ppm, acting alone, which produced an end point (MIC of Compound B).
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Q_b = concentration of compound B in ppm, in the mixture, which produced an end point.

When the sum of Q_a/Q_A and Q_b/Q_B is greater than one, antagonism is indicated. When the sum is equal to one, additivity is indicated, and when less than one, synergism is demonstrated. The lower the **SI**, the greater the synergy shown by that particular mixture. The minimum inhibitory concentration (MIC) of a microbicide is the lowest concentration tested under a specific set of conditions that prevents the growth of added microorganisms.

Synergy tests were conducted using standard microtiter plate assays with media designed for optimal growth of the test microorganism. Minimal salt medium supplemented with 0.2% glucose and 0.1% yeast extract (M9GY medium) was used for testing bacteria; Potato Dextrose Broth (PDB medium) was used for testing yeast and mold. In this method, a wide range of combinations of microbicides was tested by conducting high resolution MIC assays in the presence of various concentrations of **MI**. High resolution MICs were determined by adding varying amounts of microbicide to one column of a microtitre plate and doing subsequent ten-fold dilutions using an automated liquid handling system to obtain a series of endpoints ranging from 2 ppm to 10,000 ppm active ingredient. The synergy of the combinations of the present invention was determined against two bacteria, *Staphylococcus aureus* (*S. aureus* -- ATCC #6538) or *Pseudomonas aeruginosa* (*P. aeruginosa* -- ATCC #9027), a yeast, *Candida albicans* (*C. albicans* -- ATCC 10231), and a mold, *Aspergillus niger* (*A. niger* -- ATCC 16404). The bacteria were used at a concentration of about 5×10^6 bacteria per mL and the yeast and mold at 5×10^5 fungi per mL. These microorganisms are representative of natural contaminants in many consumer and industrial applications. The plates were visually evaluated for microbial growth (turbidity) to determine the MIC after various incubation times at 25°C (yeast and mold) or 30°C (bacteria).

The test results for demonstration of synergy of the microbicide combinations of the present invention are shown below in Tables 1 through 9. In each test, First Component (A) was **MI** and the Second Component (B) was the other commercial microbicide. Each table shows the specific combinations of

MI and the second component; results against the microorganisms tested with incubation times; the end-point activity in ppm measured by the MIC for **MI** alone (Q_A), for the second component alone (Q_B), for **MI** in the mixture (Q_a) and for second component in the mixture (Q_b); the calculated **SI** value; and the range of synergistic ratios for each combination tested (**MI**/second component or **A/B**).

Table 1

First Component (A) = 2-methyl-3-isothiazolone

Second Component (B) = benzoic acid

10

Microorganism	Q_a	Q_b	SI	A/B
<i>A. niger</i> (1 week)	0	10000	1.00	----
	50	10000	1.17	1/200
	200	10000	1.67	1/50
	300	0	1.00	-----
<i>C. albicans</i> (48 hours)	0	5000	1.00	----
	125	2000	1.03	1/16
	125	1000	0.83	1/8
	125	800	0.79	1/6.4
	125	600	0.75	1/4.8
	125	500	0.73	1/4
	125	400	0.71	1/3.2
	125	300	0.69	1/2.4
	125	200	0.67	1/1.6
	125	100	0.65	1/0.8
	125	80	0.64	1/0.64
	125	60	0.64	1/0.48
	125	50	0.64	1/0.4
	125	40	0.63	1/0.32
	150	30	0.76	1/0.2
	150	20	0.75	1/0.13
	200	0	1.00	-----
<i>S. aureus</i> (72 hours)	0	6000	1.00	----
	15	6000	1.25	1/400
	50	5000	1.67	1/100
	60	0	1.00	-----
<i>P. aeruginosa</i> (72 hours)	0	6000	1.00	----
	15	2000	1.08	1/133
	15	1000	0.92	1/67
	15	800	0.88	1/53
	15	600	0.85	1/40
	15	500	0.83	1/33
	15	400	0.82	1/27
	15	300	0.80	1/20
	20	0	1.00	-----

The synergistic ratios of **MI**/benzoic acid range from 1/0.13 to 1/67. The **MI**/benzoic acid combination showed enhanced control of Gram (–) bacteria and yeast.

Table 2

First Component = 2-methyl-3-isothiazolone

Second Component = citric acid

5

Microorganism	Q _a	Q _b	SI	A/B
<i>A. niger</i> (1 week)	0	10000	1.00	-----
	50	10000	1.17	1/200
	200	10000	1.67	1/50
	300	0	1.00	-----
<i>C. albicans</i> (72 hours)	0	10000	1.00	-----
	50	10000	1.25	1/200
	150	10000	1.75	1/67
	200	0	1.00	-----
<i>S. aureus</i> (24 hours)	0	4000	1.00	----
	15	3000	1.13	1/200
	25	2000	1.13	1/80
	25	1000	0.88	1/40
	25	800	0.83	1/32
	25	600	0.78	1/24
	25	500	0.75	1/20
	25	400	0.73	1/16
	25	300	0.70	1/12
	25	200	0.68	1/8
	40	0	1.00	-----
<i>P. aeruginosa</i> (48 hours)	0	6000	1.00	-----
	2.5	6000	1.13	1/2400
	15	5000	1.58	1/333
	20	0	1.00	-----

The synergistic ratios of **MI**/citric acid range from 1/8 to 1/40, preferably from 1/8 to 1/24. The **MI**/citric acid combinations show enhanced control of Gram (+) bacteria.

10

Table 3

Compound A = 2-methyl-3-isothiazolone

Compound B = sorbic acid

5

Microorganism	Q _a	Q _b	SI	A/B
<i>A. niger</i> (96 hours)	0	4000	1.00	-----
	50	3000	0.92	1/60
	75	3000	1.00	1/40
	75	2000	0.75	1/27
	100	3000	1.08	1/30
	100	2000	0.83	1/20
	125	3000	1.17	1/24
	125	2000	0.92	1/16
	125	1000	0.67	1/8
	150	2000	1.00	1/13
	150	1000	0.75	1/6.7
	150	800	0.70	1/5
	200	2000	1.17	1/10
	200	1000	0.92	1/5
	200	800	0.87	1/4
	300	0	1.00	-----
<i>C. albicans</i> (72 hours)	0	400	1.00	-----
	50	400	1.25	1/8
	150	300	1.50	1/2
	200	0	1.00	-----
<i>S. aureus</i> (72 hours)	0	10000	1.00	----
	40	4000	1.07	1/100
	40	3000	0.97	1/75
	50	2000	1.03	1/40
	50	1000	0.93	1/20
	50	800	0.91	1/16
	50	600	0.89	1/12
	50	500	0.88	1/10
	50	400	0.87	1/8
	50	300	0.86	1/6
	50	200	0.85	1/4
	60	0	1.00	----
<i>P. aeruginosa</i> (48 hours)	0	10000	1.00	-----
	15	3000	1.05	1/200
	15	2000	0.95	1/133
	15	1000	0.85	1/67
	20	0	1.00	-----

The synergistic ratios of **MI**/sorbic acid range from 1/4 to 1/133, preferably from 1/4 to 1/67. The **MI**/sorbic acid combination showed enhanced control of mold and both Gram (+) and Gram (–) bacteria.

Table 4

First Component = 2-methyl-3-isothiazolone

Second Component = 1,2-dibromo-2,4-dicyanobutane

5

Microorganism	Q _a	Q _b	SI	A/B
<i>A. niger</i> (1 week)	0	100	1.00	-----
	50	100	1.17	1/2
	200	100	1.67	1/0.5
	300	0	1.00	-----
<i>C. albicans</i> (72 hours)	0	500	1.00	-----
	125	200	1.03	1/1.6
	150	200	1.15	1/1.3
	150	100	0.95	1/0.7
	150	80	0.91	1/0.5
	150	60	0.87	1/0.4
	200	0	1.00	-----
<i>S. aureus</i> (72 hours)	0	1000	1.00	----
	40	800	1.47	1/20
	50	200	1.03	1/4
	50	100	0.93	1/2
	50	80	0.91	1/1.6
	60	0	1.00	-----
<i>P. aeruginosa</i> (24 hours)	0	800	1.00	-----
	5	500	0.96	1/100
	7.5	400	1.00	1/53
	7.5	300	0.88	1/40
	10	300	1.04	1/30
	10	200	0.92	1/20
	10	100	0.79	1/10
	15	0	1.00	-----

The synergistic ratios of **MI**/1,2-dibromo-2,4-dicyanobutane range from 1/0.4 to 1/100. The **MI**/1,2-dibromo-2,4-dicyanobutane combination showed enhanced control of yeast and both Gram (+) and Gram (-) bacteria.

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Table 5

First Component = 2-methyl-3-isothiazolone

Second Component = 1,3-dimethylol-5,5-dimethylhydantoin

Microorganism	Q _a	Q _b	SI	A/B
<i>A. niger</i> (1 week)	0	3000	1.00	-----
	50	3000	1.17	1/60
	200	2000	1.33	1/10
	300	0	1.00	-----
<i>C. albicans</i> (72 hours)	0	3000	1.00	-----
	50	3000	1.25	1/60
	150	3000	1.75	1/20
	200	0	1.00	-----
<i>S. aureus</i> (72 hours)	0	200	1.00	----
	15	100	0.75	1/6.7
	15	80	0.65	1/5.3
	15	60	0.55	1/4
	25	100	0.92	1/4
	25	80	0.82	1/3.2
	25	60	0.72	1/2.4
	25	50	0.67	1/2
	40	80	1.07	1/2
	40	60	0.97	1/1.5
	40	50	0.92	1/1.25
	40	40	0.87	1/1
	40	30	0.82	1/0.75
	50	40	1.03	1/0.8
	50	30	0.98	1/0.6
	50	20	0.93	1/0.4
	50	10	0.88	1/0.2
	50	5	0.86	1/0.1
	50	3	0.85	1/0.06
	60	0	1.00	-----
<i>P. aeruginosa</i> (24 hours)	0	300	1.00	-----
	2.5	200	0.83	1/80
	5	200	1.00	1/40
	5	100	0.67	1/20
	5	80	0.60	1/16
	7.5	200	1.17	1/27
	7.5	100	0.83	1/13
	7.5	80	0.77	1/11
	7.5	60	0.70	1/8
	7.5	50	0.67	1/6.7
	10	100	1.00	1/10
	10	80	0.93	1/8
	10	60	0.87	1/6
	10	40	0.80	1/4
	10	20	0.73	1/2
	10	8	0.69	1/0.8
	15	0	1.00	-----

The synergistic ratios of **MI**/1,3-dimethylol-5,5-dimethylhydantoin range from 1/0.06 to 1/80. The **MI**/1,3-dimethylol-5,5-dimethylhydantoin combination showed enhanced control of both Gram (+) and Gram (-) bacteria.

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Table 6

First Component = 2-methyl-3-isothiazolone

Second Component = phenoxyethanol

Microorganism	Q _a	Q _b	SI	A/B
<i>A. niger</i> (72 hours)	0	4000	1.00	-----
	50	3000	0.92	1/60
	50	2000	0.67	1/40
	75	3000	1.00	1/40
	75	2000	0.75	1/27
	100	3000	1.08	1/30
	100	2000	0.83	1/20
	125	3000	1.17	1/24
	125	2000	0.92	1/16
	150	2000	1.00	1/13
	300	0	1.00	-----
<i>C. albicans</i> (72 hours)	0	4000	1.00	-----
	50	3000	1.00	1/60
	75	3000	1.13	1/40
	75	2000	0.88	1/27
	100	2000	1.00	1/20
	125	2000	1.13	1/16
	125	1000	0.88	1/8
	125	800	0.83	1/6.4
	125	600	0.78	1/4.8
	125	500	0.75	1/4
	150	1000	1.00	1/6.7
	150	800	0.95	1/5.3
	150	600	0.90	1/4
	150	500	0.88	1/3.3
	150	300	0.83	1/2
	200	0	1.00	-----
<i>S. aureus</i> (72 hours)	0	4000	1.00	----
	50	2000	1.13	1/40
	60	1000	1.00	1/17
	60	800	0.95	1/13
	80	0	1.00	-----
<i>P. aeruginosa</i> (24 hours)	0	3000	1.00	-----
	2.5	2000	0.83	1/800
	5	2000	1.00	1/400
	7.5	2000	1.17	1/267
	7.5	1000	0.83	1/133

7.5	800	0.77	1/107
7.5	600	0.70	1/80
10	1000	1.00	1/100
10	800	0.93	1/80
10	600	0.87	1/60
10	400	0.80	1/40
10	300	0.77	1/30
15	0	1.00	-----

The synergistic ratios of **MI**/phenoxyethanol range from 1/2 to 1/800. The **MI**/phenoxyethanol combination showed enhanced control of Gram (+) and Gram (-) bacteria as well as yeast and mold.

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Table 7

First Component = 2-methyl-3-isothiazolone

10 Second Component = zinc pyrithione

Microorganism	Q _a	Q _b	SI	A/B
<i>A. niger</i>	0	80	1.00	-----
(1 week)	50	10	0.29	1/0.2
	75	60	1.00	1/0.8
	75	50	0.88	1/0.67
	75	40	0.75	1/0.53
	75	30	0.63	1/0.4
	75	20	0.50	1/0.27
	75	10	0.38	1/0.13
	75	8	0.35	1/0.1
	100	60	1.08	1/0.6
	100	50	0.96	1/0.5
	100	40	0.83	1/0.4
	100	30	0.71	1/0.3
	100	20	0.58	1/0.2
	100	10	0.46	1/0.1
	125	50	1.04	1/0.4
	125	40	0.92	1/0.32
	125	30	0.79	1/0.24
	125	20	0.67	1/0.16
	125	8	0.52	1/0.06
	150	40	1.00	1/0.27
	150	30	0.88	1/0.2
	150	20	0.75	1/0.13
	150	8	0.60	1/0.05
	200	30	1.04	1/0.15
	200	20	0.92	1/0.1
	200	10	0.79	1/0.05
	200	6	0.74	1/0.03
	300	0	1.00	-----

<i>C. albicans</i> (48 hours)	0	80	1.00	----
	50	60	1.00	1/1.2
	50	50	0.88	1/1
	50	40	0.75	1/0.8
	75	50	1.00	1/0.67
	75	40	0.88	1/0.53
	75	30	0.75	1/0.4
	75	20	0.63	1/0.27
	75	8	0.48	1/0.1
	100	40	1.00	1/0.4
	100	30	0.88	1/0.3
	100	20	0.75	1/0.2
	100	10	0.63	1/0.1
	100	6	0.58	1/0.06
	125	30	1.00	1/0.24
	125	20	0.88	1/0.16
	125	10	0.75	1/0.08
	125	5	0.69	1/0.04
	125	3	0.66	1/0.024
	150	20	1.00	1/0.13
	150	10	0.88	1/0.067
	150	5	0.81	1/0.033
	150	1	0.76	1/0.0067
	150	0.5	0.76	1/0.0033
	150	0.2	0.75	1/0.0013
	200	0	1.00	-----
<i>S. aureus</i> (72 hours)	0	20	1.00	----
	15	20	1.30	1/1.3
	40	20	1.80	1/0.5
	50	0	1.00	----
<i>P. aeruginosa</i> (72 hours)	0	200	1.00	----
	7.5	100	0.88	1/13
	10	100	1.00	1/10
	10	80	0.90	1/8
	15	50	1.00	1/3.3
	15	40	0.95	1/2.7
	15	30	0.90	1/2
	15	20	0.85	1/1.3
	15	10	0.80	1/0.67
	15	5	0.78	1/0.33
	15	3	0.77	1/0.2
	20	0	1.00	-----

The synergistic ratios of **MI**/zinc pyrithione range from 1/0.0013 to 1/13. The **MI**/zinc pyrithione combination showed enhanced control of Gram (-) bacteria and both yeast and mold.

Table 8

First Component = 2-methyl-3-isothiazolone

Second Component = climbazole

Microorganism	Q _a	Q _b	SI	A/B
<i>C. albicans</i> (72 hours)	0	300	1.00	-----
	50	200	0.92	1/4
	75	200	1.04	1/2.7
	75	100	0.71	1/1.3
	75	60	0.58	1/0.8
	75	40	0.51	1/0.53
	100	200	1.17	1/2
	100	100	0.83	1/1
	100	80	0.77	1/0.8
	100	60	0.70	1/0.6
	100	40	0.63	1/0.4
	100	20	0.57	1/0.2
	125	200	1.29	1/1.6
	125	100	0.96	1/0.8
	125	80	0.89	1/0.64
	125	50	0.79	1/0.4
	125	30	0.73	1/0.24
	125	10	0.66	1/0.08
	150	80	1.02	1/0.53
	150	60	0.95	1/0.4
	150	40	0.88	1/0.27
	150	20	0.82	1/0.13
	150	8	0.78	1/0.053
	200	0	1.00	-----
<i>S. aureus</i> (24 hours)	0	2000	1.00	----
	25	800	1.03	1/32
	25	600	0.93	1/24
	40	0	1.00	-----

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The synergistic ratios of **MI**/climbazole range from 1/0.05 to 1/24. The **MI**/climbazole combination showed enhanced control of Gram (+) bacteria and yeast.

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Table 9

First Component = 2-methyl-3-isothiazolone

Second Component = benzyl alcohol

Microorganism	Q _a	Q _b	SI	A/B
<i>A. niger</i> (72 hours)	0	8000	1.00	-----
	100	6000	1.08	1/60
	125	5000	1.04	1/40
	125	4000	0.92	1/32
	125	3000	0.79	1/24
	300	0	1.00	-----
<i>C. albicans</i> (48 hours)	0	6000	1.00	-----
	100	3000	1.00	1/30
	125	3000	1.13	1/24
	125	2000	0.96	1/16
	125	1000	0.79	1/8
	125	600	0.73	1/4.8
	125	400	0.69	1/3.2
	125	200	0.66	1/1.6
	125	80	0.64	1/0.64
	150	20	0.75	1/0.13
	150	2000	1.08	1/13
	150	1000	0.92	1/6.7
	150	800	0.88	1/5.3
	150	500	0.83	1/3.3
	150	200	0.78	1/1.3
	150	80	0.76	1/0.53
	150	40	0.76	1/0.27
	200	0	1.00	-----
<i>S. aureus</i> (72 hours)	0	8000	1.00	----
	40	4000	1.17	1/100
	50	2000	1.08	1/40
	50	1000	0.96	1/20
	50	800	0.93	1/16
	50	500	0.90	1/10
	50	300	0.87	1/6
	50	100	0.85	1/2
	50	50	0.84	1/1
	60	0	1.00	-----
<i>P. aeruginosa</i> (24 hours)	0	6000	1.00	-----
	2.5	5000	1.00	1/2000
	2.5	4000	0.83	1/1600
	5	4000	1.00	1/800
	5	3000	0.83	1/600
	5	2000	0.67	1/400
	5	800	0.47	1/160
	7.5	2000	0.83	1/267
	7.5	1000	0.67	1/133
	7.5	600	0.60	1/80
	10	2000	1.00	1/200
	10	1000	0.83	1/100

10	800	0.80	1/80
10	500	0.75	1/50
10	300	0.72	1/30
10	100	0.68	1/10
10	60	0.68	1/6
15	0	1.00	-----

The synergistic ratios of **MI**/benzyl alcohol range from 1/0.1 to 1/1600, preferably from 1/0.13 to 1/32 or from 1/80 to 1/1600, and more preferably from 1/80 to 1/400. The **MI**/benzyl alcohol combination showed enhanced control of

5 Gram (+) and Gram (–) bacteria as well as mold and yeast.